

1 ①. A method for preserving biological material having lipid membranes,  
2 comprising:  
3 a. Reversibly porating the lipid membranes of the biological material;  
4 b. Loading the biological material with an agent having bio-  
5 preservation properties to a predetermined intracellular concentration;  
6 c. Preparing the bio-preservation agent loaded biological material for  
7 storage;  
8 d. Storing the prepared biological material;  
9 e. Recovering the stored biological material from storage; and  
10 f. Reversing the cell membrane poration.

2. The method of claim 1, wherein the biological material comprises nucleated mammalian cells.

3. The method of claim 2, wherein the biological material is selected from the group consisting of hepatocytes, fibroblasts, chondrocytes, keratinocytes, islets of Langerhans and hematopoietic cells.

4. The method of claim 1, wherein the bio-preservation agent comprises a substantially non-permeating sugar having bio-preservation properties.

1 5. The method of claim 4, wherein the sugar having bio-preservation  
2 properties is selected from a group consisting of trehalose, sucrose, glucose,  
3 and maltose.

1 6. The method of claim 1, wherein the biological material is loaded with  
2 an intracellular concentration of bio-protective agent less than or equal to  
3 about 1.0 M.

1 7. The method of claim 6, wherein the biological material is loaded with  
2 an intracellular concentration of bio-protective agent less than or equal to  
3 about 0.4 M

1 8. The method of claim 1, wherein the lipid membranes are reversibly  
2 porated using a *Staphylococcus aureus*  $\alpha$ -toxin.

1 9. The method of claim 1, wherein the lipid membranes are reversibly  
2 porated using H5  $\alpha$ -toxin.

10. The method of claim 1, wherein the biological material is prepared for  
storage by freezing to cryogenic temperatures.

11. The method of claim 1, wherein the biological material is prepared for  
storage by freeze drying.

12. The method of claim 1, wherein the biological material is prepared for  
storage by vacuum or air drying.

13. A method for dry storing living nucleated cells, comprising:

- a. Reversibly porating the cell membranes of the nucleated cells;
- b. Loading a sugar having bio-preservation properties to a  
predetermined intracellular concentration;
- c. Drying the sugar loaded cells;
- d. Placing the dried cells in dry storage;
- e. Rehydrating the dried cells; and
- f. Reversing the cell membrane poration.

1 14. The method of claim 11, wherein the cell membranes are reversably  
2 porated using H5  $\alpha$ -toxin.

1 15. The method of claim 14, wherein the sugar having bio-preservation  
2 properties is selected from a group consisting of trehalose, sucrose, glucose  
3 and maltose.

1 16. The method of claim 15, wherein the biological material is loaded with  
2 an intracellular concentration of sugar less than or equal to about 1.0 M.

1 17. The method of claim 16, wherein the biological material is loaded with  
2 an intracellular concentration of sugar less than or equal to about 0.4 M.

1 18. The method of claim 16, wherein sugar is the only bio-protective agent  
2 employed.

1 19. The method of claim 16, wherein the drying is accomplished by freeze  
2 drying.

1 20. The method of claim 19, wherein the sugar loaded cells are plunge  
2 frozen to a cryogenic temperature.

1 21. The method of claim 16, wherein the drying is a vacuum or air drying.

1 22. The method of claim 21, wherein the drying is performed at about  
2 ambient temperature.

1 23. A method for cryopreserving nucleated cells having cell membranes,  
2 comprising:

- 3 a. Reversibly porating the cell membranes of the nucleated cells;  
4 b. Loading the porated cells with a bio-preservation agent to a  
5 predetermined intracellular concentration;  
6 c. Freezing the sugar loaded cells to a cryogenic temperature;  
7 d. Storing the frozen biological material at a cryogenic temperature;  
8 e. Thawing the cryo-stored biological material; and  
9 f. Reversing the cell membrane poration.

24. The method of claim 23, wherein the cell membranes are reversibly  
porated using a *Staphylococcus aureus*  $\alpha$ -toxin.

25. The method of claim 23, wherein the cell membranes are reversibly  
porated using H5  $\alpha$ -toxin.

26. The method of claim 23, wherein the bio-preservation agent comprises  
a substantially non-permeating sugar having bio-preservation properties.

27. The method of claim 26, wherein the sugar having bio-preservation  
properties is selected from a group consisting of trehalose, sucrose, glucose,  
and maltose.

28. The method of claim 26, wherein the biological material is loaded with  
an intracellular concentration of sugar less than or equal to about 1.0 M.

29. The method of claim 28, wherein the biological material is loaded with  
an intracellular concentration of sugar less than or equal to about 0.4 M.

1 30. The method of claim 26, wherein the sugar loaded cells are plunge  
2 frozen to a cryogenic temperature.

1 31. The method of claim 23, wherein the bio-preservation agent is a  
2 conventional penetrating cryoprotective agent.

1 32. The method of claim 31, wherein the bio-preservation agent is selected  
2 from the group consisting of DMSO, glycerol and ethylene glycol.

1 33. A method for cryopreserving living nucleated mammalian cells having  
2 cell membranes, comprising:

a. Reversably porating the cell membranes of the nucleated cells using  
H5  $\alpha$ -toxin;

b. Loading the porated cells with a bio-preservation agent consisting of  
a sugar to a predetermined intracellular concentration that is less than or equal  
to about 1.0 M;

c. Freezing the sugar loaded cells to a cryopreservation temperature;

d. Storing the frozen biological material at a cryo-storage temperature;

e. Thawing the cryo-stored biological material; and

f. Reversing the cell membrane poration.

1 34. The method of claim 33, wherein the sugar having bio-preservation  
2 properties is selected from a group consisting of trehalose, sucrose, glucose  
3 and maltose.

1 35. The method of claim 34, wherein the intracellular concentration of the  
2 sugar is less than or equal to about 0.4 M.

1 36. The method of claim 34, wherein the cells are plunge frozen.